EFFECTS OF SUBSTITUTED BENZYLPHENOLS AND SOME INSECT GROWTH REGULATORS ON THE REPRODUCTION OF FACE FLY, *Musca autumnalis* De Geer.

by

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Agron. Eng., Universidad Tecnica de Machala - Ecuador, 1978

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1984

Aproved by:
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INTRODUCTION AND LITERATURE REVIEW

The face fly, Musca autumnalis De Geer, an introduced species, is a pest of livestock and horses in southern Canada and in all of the continental United States except Florida, Louisiana, Texas, New Mexico, Arizona and Alaska (Pickens and Miller 1980). This fly annoys cattle and horses by feeding on mucous secretions of the eyes and nose. The spread of the face fly in North America has been associated with the incidence of Infectious Bovine Keratoconjunctivitis (IBK) or pinkeye disease (Cheng 1967). Face fly also is a biological vector of cattle eye worms (Thelazia spp.) (Pickens and Miller 1980), and the nematode Parafilaria bovicola, etiological agent of hemorrhagic bovine filariasis or "green muscle disease" (Bech-Nielsen et al. 1982). It has been estimated that losses in the U.S. to livestock industry due to face fly are ca. $140 million anually[1]. However, its effects on cattle weight gain has recently been disputed (Arends et al. 1982).

There is great need for improving control techniques for this pest, since no current control methods provide satisfactory results (Pickens and Miller 1980, Williams and Westby 1982). Although the face fly is susceptible to most of the insecticides commonly used for livestock pests, conventional methods have proven to be inadequate for its control. Because the face fly remains for only a short period of time on the host, the insect is not exposed to high concentrations of the insecticide (Williams and Westby 1982). Some of the methods utilized today include self-treating devices such as dust bags and oilers of

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[1] ARS, NRP No. 20480, 1977.

various designs, sprays and ear tags, none of which gives adequate control in the field. Therefore, research must be directed to the discovery of new and safer pest control strategies to ensure efficient control (Pickens and Miller 1980).

The use of sexually sterilized insects has proven to be an efficient means of controlling insect pests (Knipling 1962). Two systems can be employed when using sterility to suppress insect populations: One is the mass production, sterilization and release of sterile individuals; the other involves the direct sterilization of the natural population. Insect sterilization can be achieved by several methods including radiation, chemosterilants, cytoplasmic incompatibility, hibrid sterility and high intensity photoflash discharges (Campion 1972).

The successful use of gamma radiation for the eradication of the screwworm fly, Cochliomyia hominivorax Coquerel, has directed attention toward the possibility of controlling other species by the same method. However, such a method of inducing sterility does not seem to be applicable to all insect species (Harris 1962). Therefore, new methods for sterilizing insects are being studied. The use of chemosterilants seems to be a feasible alternative.

Chemosterilants can be used in the sterile insect technique, that is, for sterilizing the mass-reared insects before release, similar to the use of gamma radiation in the screwworm eradication (Baumhover et al. 1955, Knipling 1960). The use of chemosterilants has the added advantage that they could be applied to wild populations in the field if chemicals safe enough for this use were developed. This strategy has the obvious benefit of not having the great expense of mass rearing insects.

One of the first reports on the induction of sterility in insects by chemicals was that by Goldsmith and Frank (1952), who found that an antimetabolite affected reproduction in Drosophila spp. Since then many chemicals have been tested for their sterilizing effect on various species, especially dipterans. Campion (1972) made a detailed review of the insect chemosterilants which have been tested on species of the orders: Diptera, Coleoptera, Hymenoptera, Hemiptera, Lepidoptera and Orthoptera. The chemosterilant list included: biological alkylating agents, antimetabolites, aziridine derivatives, phosphoramides and s-triazines, organo-metals, miscellaneous compounds, insecticides and insect hormone analogues. The author concluded that the only chemosterilants that could be used as control agents are the aziridines. However, these compounds have had limited application because of their potential mutagenic and carcinogenic properties. This has led to continuous research for new types of chemosterilants which could be safely used under field conditions (Murvosh et al. 1964, Jurd et al. 1979).

Jurd et al. (1979) synthesized a series of benzylphenols and benzyl-1,3-benzodioxoles, many of which have been reported as sterilants on a number of Diptera, Lepidoptera and Coleoptera. These species include house fly, Musca domestica L. (Jurd et al. 1979, Chang et al. 1980); the screwworm fly, C. hominivorax Coquerel (Rawlins et al. 1979, Rawlins and Jurd 1981); the flesh fly, Sarcophaga bullata (Van Mellaert et al. 1982); the tsetse fly, Glossina morsitans morsitans Westwood (Langley et al. 1982); pink bollworm, Pectinophora gossypiella Saunders (Flint et al. 1980); and the Colorado potato beetle, Leptinotarsa decemlineata Say (Van Mellaert et al. 1983a). These synthetic chemoster-

ilants have been developed by molecular modification of cinnamyl-phenol and p-quinone methide, natural constituents of the Panamanian hardwood, Dalbergia retusa Henfley. These compounds are non-mutagenic and have low mammalian toxicity (Jurd et al. 1979, Jurd and Manners 1980). Subsequent reports from Van Mellaert et al. (1983b) demonstrated that some benzyl-1,3-benzodioxoles had strong and direct anti-juvenile hormone effect, and that the compounds which had increased anti-juvenile hormone activity were also good inhibitors of vitellogenesis.

Rawlins et al. (1979) found complete sterility of female screwworm flies treated with benzylphenols and benzyl-1,3-benzodioxoles. They also found the compound J2644 (2,4-bis-1,1-dimethylethyl)-6-(4-methoxyphenyl methyl)phenol) affected fertility of males, and that oral treatment with 1.0% AI of J2581 (5-ethoxy-6-(1-(4-methoxyphenyl)ethyl)-1,3-benzodioxole) proved to alter normal ovarian growth. Chang et al. (1980) observed complete sterility of females M. domestica L. when they were fed on benzylphenol, J2644.

Insect growth regulators (IGR) are gaining significance in insect pest control because of their favorable properties. The chemical structures and physiological activity of many of these IGRs may diverge considerably from those of juvenile hormone mimics, although the observed effects on target species may be quite similar (Hall and Foehse 1980, Hall et al. 1979). In general, the precise mode of action with respect to many recently synthesized compounds remains poorly understood.

The experimental IGR, BAY SIR 8514 (2-chloro-n-[[[4-(trifluoromethoxy)phenyl]amino]carbanyl]benzomide) has shown considerable promise for pest control especially against dipterous species (Cantelo 1979, Ali

and Lord 1980, Weaver and Begley 1982, Johnson and Mulla 1982). This compound has been reported to cause not only abnormal development of immature stages but also abnormal hatching and ovicidal activity on mosquitoes (Shaefer et al. 1978, Miura and Takahashi 1979). Sterility has been induced in female house flies for as long as 35 days when flies were injected with BAY SIR 8514 (Chang 1979). Weaver and Begley (1982) found a significant degree of sterility in house flies for up to 21 d after oral treatments; however, the effect lasted only 14 d when flies were exposed to contact treatments. They suggested that this compound acts as a chitin synthesis inhibitor and that the effects are similar to those produced by diflubenzuron, reported to cause inhibition of egg hatch in face fly by oral or tarsal treatments (Pickens and De Milo 1977, Knapp 1982). More recently, Knapp and Herald (1983) found great inhibition of egg hatch in face flies exposed to surfaces treated with BAY SIR 8514. They also found that the sterilant effect of this compound is greatest in female flies. The IGR CGA-72662 (N-cyclopropyl-1,3,5-triazine-2,4,6- triamine) and analogous compounds have been tested mainly on immature stages of dipterans (Hall et al. 1979, Hall and Foehse 1980). Treatments of adult house flies by Hall and Foehse (1980) gave no sterilant effect when the chemical was administered in water.

Ro13-5223 (ethyl(p-phenoxyphenoxy)ethyl)carbamate) is one of a series of carbamate-type compounds which have exhibited juvenile hormone activity. This compound has been evaluated for insect growth regulating activity on several stored-product insects, and found to be effective in preventing the development of nine coleopteran and three lepidopteran species (Kramer et al. 1981).

Chemosterilization of face fly was first demonstrated by Hair and Adkins (1964) using apholate and tepa. Subsequently, various compounds have been tested as sterilants upon face fly (Zapanta and Wingo 1968, Kaur and Steve 1969, Dharm and Steve 1969, Lang and Treece 1971). However, none of the reported chemosterilants have had a practical application (Campion 1972).

The present work was conducted to evaluate the sterilizing effect of benzylphenols, benzyl-1,3-benzodioxoles and IGRs upon the face fly. This research was undertaken also to provide information about the effects of these new chemosterilants which, as stated by Van Mellaert et al. (1983b), could bring a new step closer to the practical realization of what has been called "the fourth generation" of the pest control principle (Bowers et al. 1976, Van Mellaert et al. 1983b). Specific objectives for this work were: (1) to document the chemosterilant effect of the benzylphenol J2644 , benzyl-1,3-benzodioxoles (J2922 and J2581), and the IGRs BAY SIR 8514, CGA-72662 and Rol3-5223 on face fly; (2) to characterize the efficacy associated with mode of application; (3) to determine the chemosterilant effect on both sexes of face flies; and (4) to determine if the induced sterility is temporary or permanent.

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PAPER

EFFECTS OF SUBSTITUTED BENZYLPHENOLS AND SOME INSECT GROWTH REGULATORS ON THE REPRODUCTION OF FACE FLY, *Musca autumnalis* De Geer

ABSTRACT

New, non-mutagenic chemosterilants, a benzylphenol (J2644) and two benzyl-1,3-benzodioxoles (J2922 and J2581), and the insect growth regulators (IGR) BAY SIR 8514, CGA-72662 and Rol3-5223 were evaluated for their sterilizing effects on the face fly Musca autumnalis De Geer. J2644, J2922, J2581 and BAY SIR 8514 prevented egg hatching when flies were treated orally, topically or tarsally. J2644 and BAY SIR 8514 were not toxic at doses giving 100% inhibition of egg hatching and caused sterility even when flies were inseminated or near oviposition. Effects of J2922 and J2581 were similar in that their lowest doses giving maximum sterility were also highly toxic. Rol3-5223 was not effective at any of the tested concentrations (0.1 to 1.0% AI) administered orally or topically, nor was CGA-72662 applied orally.

J2644, J2922, J2581 and BAY SIR 8514 caused 100% sterility in females, whereas male fertility was only slightly reduced with J2644. The female sterility induced by these compounds was reversible. Flies treated with J2644, J2922 and J2581 restored their normal fertility by the 3rd gonadotrophic cycle, whereas flies treated with BAY SIR 8514 restored fertility by the 4th cycle. J2922 and J2581 affected egg hatching and ovarian development, observed as atrophied ovaries and ovaries containing eggs without respiratory mast, this effect was greater in the 2nd gonadotrophic cycle subsequent to the treatment. The effects on ovarian development observed with J2922 and J2581 may be useful in future physiological research and may help in understanding the mode of action of these compounds.

EFFECTS OF SUBSTITUTED BENZYLPHENOLS AND SOME INSECT GROWTH REGULATORS ON THE REPRODUCTION OF FACE FLY, Musca autumnalis De Geer

Many compounds have been evaluated for chemosterilizing effects after the first report of chemical sterilization on insects was published (Goldsmith and Frank 1952). However, many of the chemicals which have been reported as chemosterilants have not had practical application in insect pest control because they appear to be toxic and mutagenic to non-target organisms (Campion 1972). Jurd et al. (1979) reported the benzyl-phenols and benzyl-1,3-benzodioxoles had high chemosterilant activity with low mammalian toxicity and were non-mutagenic. A considerable number of these compounds were found to sterilize house flies, Musca domestica L. Subsequent studies have demonstrated that some compounds of this series affected reproduction in other species. These include the screwworm fly, Cochliomyia hominivorax Coquerel (Rawlins et al. 1979); tsetse fly, Glossina morsitans morsitans Westwood (Langley et al. 1982); the flesh fly, Sarcophaga bullata (Van Mellaert et al. 1982); pink bollworm, Pectinophora gossypiella (Flint et al. 1980); and the Colorado potato beetle, Leptinotarsa decemlineata Say (Van Mellaert et al. 1983a). Most of the compounds of this series have been shown to sterilize females and some have been reported to affect male reproduction. However, Chang et al. (1980) found that they have lower activity against male house flies. It has also been shown that some of these compounds have strong and direct anti-juvenile hormone activity and that such activity is correlated with sterilant effects (Van Mellaert et al. 1982, De Loof and Van Mellaert 1982, Van Mellaert et al. 1983b).

Recent reports have indicated that some insect growth regulators such as diflubenzuron, penfluron, and BAY SIR 8514 also have sterilizing effect on dipterans (Chang 1979; Shaefer et al. 1978, Weaver and Begley, 1982). More recently Knapp and Herald (1983) reported sterilizing effects of BAY SIR 8514 when adult face flies were exposed to residues of this compound. The work herein reported evaluated the chemosterilant effects of the benzylphenol J2644, benzyl-1,3-benzodioxoles J2922 and J2581, and the IGRs BAY SIR 8514, CGA-72662, and Rol3-5223 on face flies. These compounds were evaluated for their sterilant activity in relation to mode of application, permanency of effects and activity on both sexes.

MATERIALS AND METHODS

Face flies, obtained from a colony established in September, 1982, with flies collected in Linn Co. KS, were held in screening cages and maintained at 25±2°C and 60±5% RH, with a 14:10 L:D photoperiod. Flies were provided with sugar, water, and a protein source (powdered milk and powdered egg).

Compounds evaluated included:

J2644: 2,4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol

J2922: 5-ethoxy-6-[1-(4-methoxyphenyl)ethyl]-1,3-benzodioxole

J2581: 5-ethoxy-6-(4-methoxyphenylmethyl)-1,3-benzodioxole, supplied by L. Jurd, Western Regional Research Center, Agric. Res., SEA, USDA. Berkeley, CA.

BAY SIR 8514: 2-chloro-N-[[[4-(tryfluoromethoxy)phenyl]amino]carbaryl] benzamide supplied by Mobay Chem.Corp. Agric. Chem. Div. Kansas City, MO.

CGA-72662: N-cyclopropyl-1,3,5-triazine-2,4,6-triamine, supplied by CIBA GEIGY, Agric. Div. Greensboro, NC.

Ro13-5223: Ethyl[2-(p-phenoxyphenoxy)ethyl]carbamate, supplied by MAAG Agrochemicals, HLR Sciences, Inc., Vero Beach, FL.

Treatments

Oral.-Chemicals were dissolved in acetone in eight concentrations ranging from 0.01 to 1.0%, of which 100 ml were added to 100 g of powdered sugar. After acetone evaporation the dry powder was ground in a mortar. The compound CGA-72662 is insoluble in acetone, thus it was added directly to sugar to make a stock mixture of 1% (w/w). It was subsequently diluted to desired concentrations by the addition of sugar. Shortly after eclosion, 25 flies of each sex were caged together and fed ad libitum the treated sugar for 2 d, after which they were fed pure sugar. Water and diet were also added to the cages. This test was replicated 4 times per concentration.

Topical.-Four groups of flies, similar to the previous test, were treated topically with 2 µl of one of 5 acetone solutions of the compounds, ranging from 0.01 to 1.0%. Solutions were applied to the venter of the abdomen to CO_2-anesthetized flies. Controls were treated with acetone alone. After treatment all flies were held in cages with pure sugar, protein source and water. CGA-72662 was not used on this test due to its low acetone solubility.

Tarsal.-This treatment was performed following the techniques of Rawlins and Jurd (1981). About 10 ml of the same acetone solutions as used in the topical applications were poured into each one-quart wide mouth jar. Jars were rotated and then emptied, allowed to dry and left

open for 24 h. Petri dishes, used to cover the jars, were treated accordingly. Batches of 1-day-old flies, as before, were held in the jars for 2 h before being transferred to holding cages. Each treatment was replicated 4 times. CGA was not used in this test either.

Sterility determination.- When flies were 7 days old, they were offered fresh bovine dung in small petri dishes as oviposition medium. After 6 h, the dung was removed and 50 eggs were collected at random and transferred onto moistened filter paper in petri dishes. Eggs were kept in the same room as flies. Egg hatching was recorded 24 h later. Adult mortality was also recorded at the day of oviposition. The sterilizing effect of each dose was expressed in terms of percent inhibition of egg hatching (X), where X = 100 - [(percent hatch of treated group/ percent hatch of control group) X 100], Rawlins et al. (1979).

Sterilant effect at different time of exposure

The lowest doses giving highest sterility and low toxicity of the most potent sterilants from previous experiments were administered orally for varying periods of 1 to 5 d to determine the effects of different time of exposure on them. Groups of 20 newly emerged flies of each sex were fed the treatment for the corresponding time, after which the treated sugar was replaced by pure sugar for the rest of the experiment. Each treatment was replicated 4 times. On the 7th day oviposition medium was provided and inhibition of egg hatching and adult mortality was determined.

J2644 (1.0%) and BAY SIR 8514 (0.05%) were tested to determine the effects of a single meal administered at different intervals after adult emergence (2, 4, and 6 d). Groups of 25 flies of each sex emerging

within 6 h were used for this experiment. Flies previously starved for 24 h were fed for 2 h the treated food at the respective time after adult emergence. Pure sugar was provided for the rest of the experiment. Four replications of each treatment were used. On the same day of exposure, 40 females of the same age as the exposed flies were dissected to determine the stage of ovarian development and insemination, following the techniques of Van Geem et al. (1983). On the 7th day oviposition medium was provided to determine inhibition of egg hatching .

Sterilizing effect on each sex

Non-lethal concentrations (i.e., J2644 1.0%; J2922 and J2581 at 0.1%; and BAY SIR 8514 0.05%) were administered orally for the first 5 days after adult eclosion to determine their effects on each sex. Groups of 40 newly emerged flies were separated by sex and fed the treatment which was replaced by normal sugar on the 5th day, after which 40 flies of each sex and of the same age were mixed to make up the following mating:

Untreated male x Untreated female (control)

Untreated male x Treated female

Treated male x Untreated female

Treated male x Treated female

Each treatment was replicated 4 times. On the 7th day inhibition of egg hatching was determined as previously. On the same day and before egging, a sample of 40 females (10 from each replication) were dissected to determine the number of eggs per female. A similar sample was dissected immediately after egging to determine the percent of females inseminated and ovipositing.

Techniques followed were described by Van Geem et al. (1983).

Permanency of Induced Sterility

The compounds giving high sterilizing effects in previous tests were tested orally at non-toxic concentrations to determine the reversibility of the induced sterility on subsequent gonadotrophic cycles. Groups of 50 newly emerged flies of each sex were fed for 5 d with the following treatments: J2644 (1.0%); J2922 and J2581 (0.1%); and BAY SIR 8514 (0.05%). Each treatment was replicated 4 times. After the 5th day flies were provided with pure sugar and on days 7, 14, 21, and 28 flies were egged and inhibition of egg hatching determined as before.

J2922 and J2581 affected ovarian development, thus a test was done to study these compounds on different gonadotrophic cycles. Groups of 200 flies of each sex were caged and fed a 0.1% concentration of these compounds . One group was treated for the first 5 days after eclosion (nulliparous flies), and a second group treated for 5 d after the first oviposition (uniparous flies). Each treatment was replicated 4 times. Examination of the ovaries was done at the time that flies were expected to be gravid, (i.e., 7, 14, 21, and 28 d after emergence). Forty females from each treatment were dissected to determine any abnormal ovarian development as indicated by atrophied ovaries or eggs without respiratory masts.

RESULTS AND DISCUSSION

Mode of administration

Toxic and chemosterilant activity of oral administration of the tested compounds are shown in Table 1. Flies tolerated 1.0% concentrations of J2644, CGA-72662 and Ro13-5223. High adult mortality, however, was observed with J2922, J2581 and BAY SIR 8514 at high concentrations. Non-lethal dosages of J2922 and J2581 caused only ca. 25% sterility while concentrations needed to produce high sterility were also highly toxic. J2644 and BAY SIR 8514 caused 100% egg sterility at the non-lethal concentrations of 1.0 and 0.05%, respectively. The effects of J2644, J2922, J2581 and BAY SIR 8514 on sterility were dose-dependent. CGA-72662 and Ro13-5223 did not give high sterility at any of the tested concentrations, and in fact, response was not dose-dependent.

Effects of topical applications are shown in Table 2. J2644 did not affect adult survival even at the highest dosage of 1.0%, but egg hatch was inhibited completely at this concentration. J2922 and J2581 were very toxic at 1.0% concentration, and sterility values of 62 and 95% were obtained with 0.75 and 1.0% of J2922 and J2581, respectively. J2922, at 1.0%, caused 91% mortality, thus sterility at this concentration was not determined. BAY SIR 8514 was not toxic at concentrations lower than 0.05%, however, 100% sterility was achieved with concentrations as low as 0.075%. Ro.13-5223 did not affect either survival or egg hatchability.

When test compounds were applied tarsally, only BAY SIR 8514 gave 100% inhibition of egg hatching (Table 3); this level was obtained with concentrations as low as 0.1%. These results are similar to those

reported recently by Knapp and Herald (1983), who found 100% egg sterility in face flies exposed to surfaces treated with BAY SIR 8514. Weaver and Begley (1982) also obtained complete sterility of house flies treated tarsally with this compound. J2644 was not toxic to adults at any of the tested concentrations; however, the sterilant effect of this compound increased with dosage to 79% sterility at 1.0% AI. These results are similar to those reported by Rawlins and Jurd (1981), who obtained 80 to 100% sterility when 3 to 5-day-old screwworm flies were exposed to residues deposited by 1.0% of J2644. J2922 and J2581 had similar effects on adult mortality and on egg hatchability. The highest doses caused up to 72% adult mortality and up to 65% inhibition of egg hatching.

Results of these series of tests to determine effects of mode of application indicated that BAY SIR 8514 and J2644 are not toxic at the lowest doses producing 100% inhibition of egg hatch; however, this minimum dose for BAY SIR 8514 was 20X lower than that for J2644. Oral and topical treatments resulted in similar dose-effect relationship; however, no dose-effect relationship was observed in tarsal treatments. Effects of J2922 and J2581 were also similar in that their lowest doses giving maximum sterility were also highly toxic. The mortality and sterility responses were linear and highly correlated. Ro13-5223 was not effective at any of the tested concentrations administered orally and topically, nor was CGA-72662 applied orally.

Effects of the period of exposure

When both sexes were treated with J2644 (1.0%) or BAY SIR (0.05%) during the first day after adult emergence, 96 and 100% inhibition of

egg hatching occurred with each compound, respectively (Table 4). These compounds fed for more than 1 d caused 100% sterilization. J2922 and J2581, at non-lethal dosages (0.10%), needed at least 4 d of exposure to cause 90 to 100% sterility. Significant linear correlations ($r^2 > 0.93$) were observed between exposure time and sterility in treatments with both of the latter two compounds. No relationship could be observed with the former compounds as the dose used gave ca. 100% sterility with all exposures used.

The effects of J2644 (1.0%) and BAY SIR 8514 (0.05%) on mixed sexes exposed for a single meal after 24 h of starvation (Table 5) indicated that these two compounds are more efficient when sterilizing face flies in advanced gonadotrophic stages than in earlier stages and that they can sterilize females even when they are already inseminated and near stage 5, i.e., the ovipositing stage.

Effects of the chemosterilants on each sex

All 4 compounds evaluated for their effect on each sex (J2644, 1.0%; J2922, 0.1%; J2581, 0.1%; BAY SIR 8514, 0.05%) caused 100% inhibition of egg hatching when females treated for the first 5 days after emergence were mated with untreated males of the same age (Table 6). Similar results were observed when treated females were mated with treated males. On the other hand, the chemosterilant effect on males was not significant as demonstrated by low inhibition of egg hatch from untreated females mated with treated males; J2644 gave the highest percent male sterility (29%). Chang et al. (1980) reported that J2922 and J2644 were effective sterilants of female house flies, but J2644 only slightly affected male sterility. These results could also be

compared to those reported by Rawlins et al. (1979), who found J2644 and J2581 affecting mainly female screwworm flies.

The compounds tested did not affect mating (i.e., insemination or oviposition), except when J2922 and J2581 were applied to females alone or mixed sexes and a reduction in the number of ovipositing females was observed (See Table 6). This reduction was related to the presence of females with atrophied ovaries and with abnormal eggs, i.e., eggs without respiratory masts. Abnormal eggs viewed under a scanning electron microscope (ETEC Autoscan U-1) indicated to lack respiratory mast and meshwork, in addition to abnormalities in the development and position of the micropyle (See Fig. 2 A - F). The mean number of eggs per female in the first ovipositing period was also significantly reduced (19 to 20 per female) when flies were treated with J2922 or J2581, while control flies had 22 eggs.

Permanency of the induced sterility

When mixed sexes of flies were treated with the test compounds, J2644 (1.0%), J2922 (0.10%), J2581 (0.10%) and BAY SIR 8514 (0.05%) during the first 5 days after emergence, all chemicals reduced egg hatchability by 96 to 100% in the 1st and 2nd egging cycles (7-14 d after adult emergence), (Table 7). However, flies treated with J2644, J2922 or J2581 recovered their normal fertility by the 3rd egging cycle (21 d after adult emergence). Flies treated with BAY SIR 8514 were still sterile by the 3rd cycle; however, normal fertility also was restored by the 4th cycle. This indicates the sterilant effect of these compounds is reversible. Similar reversible sterility has been reported on house flies treated with J2644 (Chang et al. 1980) and with BAY SIR 8514

(Weaver and Bebley 1982).

J2922 and J2581 affected not only egg hatchability but also ovarian development. When these compounds were fed (0.10%) for 5 d to nulliparous or uniparous flies, they affected ovarian development (atrophied avaries and ovaries containing eggs without respiratory mast). Their effect on ovarian development was observed mostly by the 2nd gonadotrophic cycle subsequent to treatment (Fig 1), when 60 and 42% of the ovaries were found abnormal in flies treated with J2922 and J2581, respectively, after being only 32 and 17%, in the first cycle. Ovaries in the 3rd cycle were not affected. A similar pattern was observed in flies treated after the first oviposition in that the highest rate of ovarian abnormalities was observed in the 3rd cycle (2nd cycle after treatment).

The greater effect of J2922 and J2581 on ovarian development on the second gonadotrophic cycle after treatment could be due to the fact that face fly ovaries exhibit meroistic development, that is, each ovariole contains both oocytes and nurse cells of the polytrophic subtype (Van Geem et al. 1983) and these chemicals mostly affected early stages of ovarian development. The observed effects of J2922 and J2581 could be useful in future studies on understanding the mode of action of these compounds. De Loof and Van Mellaert (1982) and Van Mellaert et al. (1983b) reported these compounds to have an anti-juvenile hormone activity. Whatever the mode of action, the sterilant activity and the effects on ovarian development make these chemicals good candidates to be used in subsequent physiological research.

Chang et al. (1980) suggested that since treated flies recover fertility, this class of chemosterilants has little potential for

controlling fly populations. It is obvious this type of chemicals has little potential in the sterile-insect release technique; however, even if the produced sterility is reversible, these chemicals can be used when applied continuosly (as baits or residues) to natural populations. This fact has been demostrated by Rawlins et al. (1982) who found reduction of confined house fly populations to minimal levels within 3 to 5 wks after initiation of treatment with J2644 as a bait.

Table 1.-Chemosterilant and toxic effects of benzylphenol, benzyl-1,3-benzodioxoles and insect growth regulators on mixed sexes of face flies treated orally.[1]

CHEMICAL	Conc. (%)	Adult mortality[2] (%) X ± SD	Inhibition of egg hatching[3] (%) X ± SD
J2922	0.025	0.8 ± 1.5	13.4 ± 5.0
	0.050	1.9 ± 1.7	9.79 ± 4.2
	0.075	0.5 ± 0.6	19.58 ± 3.8
	0.10	0.8 ± 1.5	24.22 ± 7.4
	0.25	57.2 ± 5.4	53.61 ±11.0
	0.50	86.5 ± 8.0	----------
	0.75	97.4 ± 3.1	----------
	1.0	100	----------
J2581	0.025	0.9 ± 1.8	1.31 ± 2.6
	0.050	2.3 ± 2.8	3.97 ± 7.9
	0.075	1.3 ± 1.7	6.31 ± 1.2
	0.10	0.8 ± 0.9	26.84 ±14.1
	0.25	18.8 ± 8.4	33.68 ±14.3
	0.50	41.9 ± 8.9	51.57 ± 9.2
	0.75	72.8 ± 8.7	100
	1.0	99.5 ± 1.0	----------
J2644	0.025	1.3 ± 1.5	13.40 ± 6.1
	0.050	0.8 ± 1.2	32.47 ± 9.9
	0.075	1.8 ± 2.3	37.63 ± 4.5
	0.10	1.9 ± 2.1	71.39 ± 7.6
	0.25	1.4 ± 2.2	82.73 ±10.2
	0.50	0.1 ± 0.3	92.27 ± 5.4
	0.75	0.8 ± 1.2	96.40 ± 4.2
	1.0	1.3 ± 2.3	100
BAY SIR 8514	0.010	0.5 ± 0.6	16.32 ± 8.1
	0.025	1.5 ± 2.4	76.38 ± 8.9
	0.050	1.5 ± 2.4	100
	0.075	11.8 ± 5.1	100
	0.10	33.5 ± 7.2	100
	0.25	88.1 ±10.1	----------
	0.50	100	----------
	0.75	100	----------
	1.0	100	----------

Cont.....

Table 1.-(Cont.)

CHEMICAL	Conc. (%)	Adult mortality[2] (%) X ± SD	Inhibition og egg hatching[3] (%) X ± SD
CGA-72662	0.025	0.4 ± 0.8	7.5 ± 5.0
	0.050	0.9 ± 1.8	9.3 ± 6.5
	0.075	0.4 ± 0.8	15.0 ± 3.3
	0.10	0.8 ± 0.9	15.0 ± 5.1
	0.25	0.4 ± 0.8	18.6 ± 9.2
	0.50	1.3 ± 1.7	17.7 ± 6.2
	0.75	0.9 ± 1.8	16.6 ± 5.2
	1.0	0.8 ± 0.9	19.2 ± 6.1
Rol3-5223	0.025	2.0 ± 2.9	5.5 ± 4.5
	0.050	0.5 ± 1.0	2.9 ± 4.9
	0.075	1.5 ± 1.9	5.5 ± 5.7
	0.10	2.5 ± 3.1	6.5 ± 6.2
	0.25	2.0 ± 2.9	10.2 ± 3.9
	0.50	0.5 ± 1.0	13.9 ± 4.7
	0.75	1.5 ± 1.9	20.8 ± 6.6
	1.0	2.0 ± 2.9	18.7 ± 5.5

[1] Chemicals in powder sugar administered for 2 d.

[2] %adult mortality = 100 - [(%survivals in the experimental group)/(%survivals in the control group) X 100].

[3] %inhibition of egg hatching = 100- [(%hatching in treatment group)/(%hatching in control group) X 100].

Table 2.-Chemosterilant and toxic effects of benzylphenol, benzyl-1,3-benzodioxoles and insect growth regulators on mixed sexes of face flies treated topically.[1]

CHEMICAL	Conc. (%)	Adult mortality[2] (%) X ± SD	Inhibition of egg hatching[3] (%) X ± SD
J2922	0.10	5.8 ± 3.0	14.7 ± 9.6
	0.25	15.7 ± 3.6	29.3 ± 6.5
	0.50	55.5 ± 7.5	69.6 ± 6.1
	0.75	78.0 ± 2.7	61.9 ±11.4
	1.0	90.6 ± 4.0	---------
J2581	0.10	0.9 ± 1.1	4.5 ± 1.5
	0.25	1.9 ± 2.1	6.1 ± 4.1
	0.50	10.7 ± 3.5	8.1 ± 4.6
	0.75	49.7 ±11.3	22.0 ± 4.9
	1.0	77.7 ± 7.4	94.9 ± 6.7
J2644	0.10	0.8 ± 1.5	3.6 ± 4.9
	0.25	1.0 ± 1.5	15.6 ±10.4
	0.50	0.7 ± 0.5	31.8 ± 8.6
	0.75	1.3 ± 1.3	81.3 ±10.3
	1.0	1.3 ± 2.6	100
BAY SIR 8514	0.010	00	16.7 ± 5.4
	0.025	00	49.8 ±12.83
	0.050	2.7 ± 1.1	98.4 ± 3.2
	0.075	14.5 ± 7.0	100
	0.10	31.5 ± 3.4	100
	0.25	42.5 ± 3.4	100
	0.50	80.5 ± 7.0	---------
Ro13-5223	0.10	1.0 ± 1.2	0.5 ± 1.0
	0.25	0.5 ± 1.0	1.6 ± 2.0
	0.50	1.5 ± 1.9	4.2 ± 5.2
	0.75	1.5 ± 1.9	3.7 ± 3.6
	1.0	1.0 ± 2.0	15.9 ± 5.7

[1] Chemicals administered in acetone solutions.

[2] % adult mortality = 100 - [(%survivals in the experimental group)/(%survivals in the control group) X 100].

[3] %inhibition of egg hatching = 100 - [(%hatching in treatment group)/(%hatching in control group) X 100].

Table 3.-Chemosterilant and toxic effects of benzylphenol, benzyl-1,3-benzodioxoles and BAY SIR 8514 on mixed sexes of face flies treated tarsally.[1]

CHEMICALS	Conc. (%)	Adult mortality[2] (%) X ± SD	Inhibition of egg hatching[3] (%) X ± SD
J2922	0.10	0.9 ± 1.1	6.9 ± 6.3
	0.25	8.3 ± 3.5	11.7 ± 6.1
	0.50	42.0 ± 7.7	34.0 ±12.2
	0.75	64.2 ± 4.6	37.8 ±14.6
	1.0	72.0 ± 8.0	65.4 ± 9.2
J2581	0.10	0.4 ± 0.8	0.9 ± 1.2
	0.25	5.6 ± 2.9	1.9 ± 2.3
	0.50	8.7 ± 2.6	4.9 ± 3.9
	0.75	42.6 ± 4.4	22.7 ±13.1
	1.0	63.1 ± 7.7	57.3 ±10.3
J2644	0.10	0.5 ± 1.0	4.9 ± 4.2
	0.25	1.5 ± 1.9	18.7 ± 6.8
	0.50	1.5 ± 1.9	46.0 ±12.3
	0.75	1.0 ± 2.0	49.2 ±10.2
	1.0	1.5 ± 1.9	79.1 ± 9.1
BAY SIR 8514	0.010	2.5 ± 3.3	14.4 ± 9.4
	0.025	3.8 ± 3.8	37.4 ± 8.1
	0.050	3.5 ± 2.5	74.9 ± 5.3
	0.075	16.7 ± 5.0	96.2 ± 6.1
	0.10	27.3 ± 8.2	100
	0.25	43.9 ± 7.6	100
	0.50	55.0 ± 9.5	100

[1] Flies exposed to residues of the compounds for 2 h.

[2] %adult mortality = 100 - [(%survivals in the experimental group)/(%survivals in the control group) X 100].

[3] %inhibition of egg hatching = 100 - [(%hatching in treatment group)/(%hatching in control group) X 100].

Table 4.- Chemosterilant activity of benzylphenol, benzyl-1,3-benzodioxoles and BAY SIR 8514 on mixed sexes of face flies treated orally[1] for varying periods after emergence.

Exposure	J2922		J2581		J2644		BAY SIR 8514	
period (days)	%adult mortality[2]	%Inhibition egg hatch[3]	%adult mortality	%inhibition egg hatch	%adult mortality	%nhibition egg hatch	%adult mortality	%inhibition egg hatch
1	00	2.7± 2.5[4]	1.0±1.4	9.5±11.4[4]	1.5±2.4	95.8±5.5	1.2±2.5	100
2	2.5±2.4	16.7±10.0	0.2±0.5	25.1±22.6	0.8±1.5	100	1.5±1.9	100
3	0.2±0.5	46.9±18.3	3.1±3.0	54.4±28.6	1.8±2.0	100	4.6±4.2	100
4	1.5±1.9	97.9± 4.2	6.1±2.6	90.0±14.8	2.3±2.7	100	18.4±13.0	100
5	6.4±2.8	96.3± 7.3	7.9±6.4	100	5.9±4.6	100	15.8±5.2	100

[1] Chemicals administered in powder sugar at the dosages of: J2644 (1.0%), J2922 (0.10%), J2581 0.10%) and BAY SIR 8514 (0.05%).

[2] %adult mortality = 100-[(%survivals in tyreatment group)/(%survivals in control group)]X 100]± SD.

[3] %inhibition of egg hatching = 100-[(%hatch in treatment group)/(%hatch in control group) X 100]± SD.

[4] Significant linear corelations ($r^2 > 0.93$) were obserbed between exposure time and sterylity.

Table 5.-Effects of J2644 (1.0%) and BAY SIR 8514 (0.05%) on mixed sexes of face flies at different stages of development and offered treated food for 2 h after 24 h of starvation.

Treatment day[1]	Physiol. and mating stages		Inhibition of egg hatching[3] (%) X ± SD	
	Physiol. stage[2]	% mated	J2644	BAY SIR 8514
2	0.1 ± 0.6	00	74.9 ± 16.2	83.6±17.3
4	3.0 ± 0.9	55.0	100	96.3± 7.5
6	4.7 ± 0.5	92.5	93.9 ± 8.3	100

[1] Days after adult emergence.

[2] Physiological stage according to Van Geem et al. (1983).

[3] %inhibition of egg hatching = 100 - [(%hatching in treatment group)/(%hatching in control group) X 100].

Table 6.-Chemosterilant activity of benzylphenol[1], benzyl-1,3-benzodioxoles[2] and BAY SIR 8514[3] on each sex of face flies treated orally for the first 5 days after emergence.

TREATMENT		Mean % flies[4]		Mean No. eggs/female	Inhibition of egg hatching[5] (%) X ± SD
Female	Male	Ovipositing	Inseminated		
UT[6]	UT	98.7 ab	100 a	22.3 a	00
UT	J2922	97.5 ab	100 a	22.6 a	9.0 ± 8.7
J2922	UT	72.5 e	87.5 c	19.0 c	100
J2922	J2922	80.0 de	92.5 ab	19.5 bc	100
UT	J2581	93.7 abc	96.2 ab	22.0 a	16.3 ± 7.2
J2581	UT	88.7 bcd	95.0 ab	20.3 b	100
J2581	J2581	85.0 cd	98.7 a	19.8 bc	100
UT	J2644	100 a	100 a	22.4 a	28.9 ± 7.7
J2644	UT	96.2 ab	98.7 a	22.0 a	100
J2644	J2644	97.5 ab	98.7 a	22.5 a	100
UT	SIR 8514	96.2 ab	100 a	22.6 a	7.6 ± 5.4
SIR 8514	UT	98.7 ab	100 a	22.5 a	100
SIR 8514	SIR 8514	97.5 ab	100 a	22.7 a	100

[1] J2644 (1.0%).
[2] J2922 and J2581 (0.10%).
[3] BAY SIR 8514 (0.05%).

[4] Means with the same letter are not significantly different, LSD, ($P > 0.05$).

[5] %inhihition of egg hatching = 100 - [(%hatching in treatment group)/(%hatcvhing in control group) X 100].

[6] UT= untreated.

Table 7.-Lasting effects of benzylphenol[1], benzyl-1,3-benzodioxoles[2] and BAY SIR 8514[3] on the reproduction of face flies treated orally for 5 days after emergence.

Day of oviposition	Gonadotrophic cycle	Inhibition of egg hatching[4] (%) X ± SD			
		J2922	J2581	J2644	BAY SIR 8514
7	1	100	98.9 ± 2.2	100	100
14	2	96.8 ± 6.5	100	100	100
21	3	7.6 ± 8.0	16.8 ± 12.5	16.8 ±12.1	95.6 ± 6.1
28	4	00	2.9 ± 5.8	2.9 ± 5.8	11.0 ± 9.9

[1] J2644 (1.0%).
[2] J2922 and J2581 (0.1)%).
[3] BAY SIR 8514 (0.05%).

[4] %inhibition of egg hatching = 100 - [(%hatching in treatment group)/(%hatching in control group).

Figure 1.-Percent of female face flies with abnormal ovarian development (atrophied ovaries and ovaries containing eggs without respiratory mast) after treatment with J2922 and J2581 (0.1% AI) when flies were in different gonadotrophic cycles.

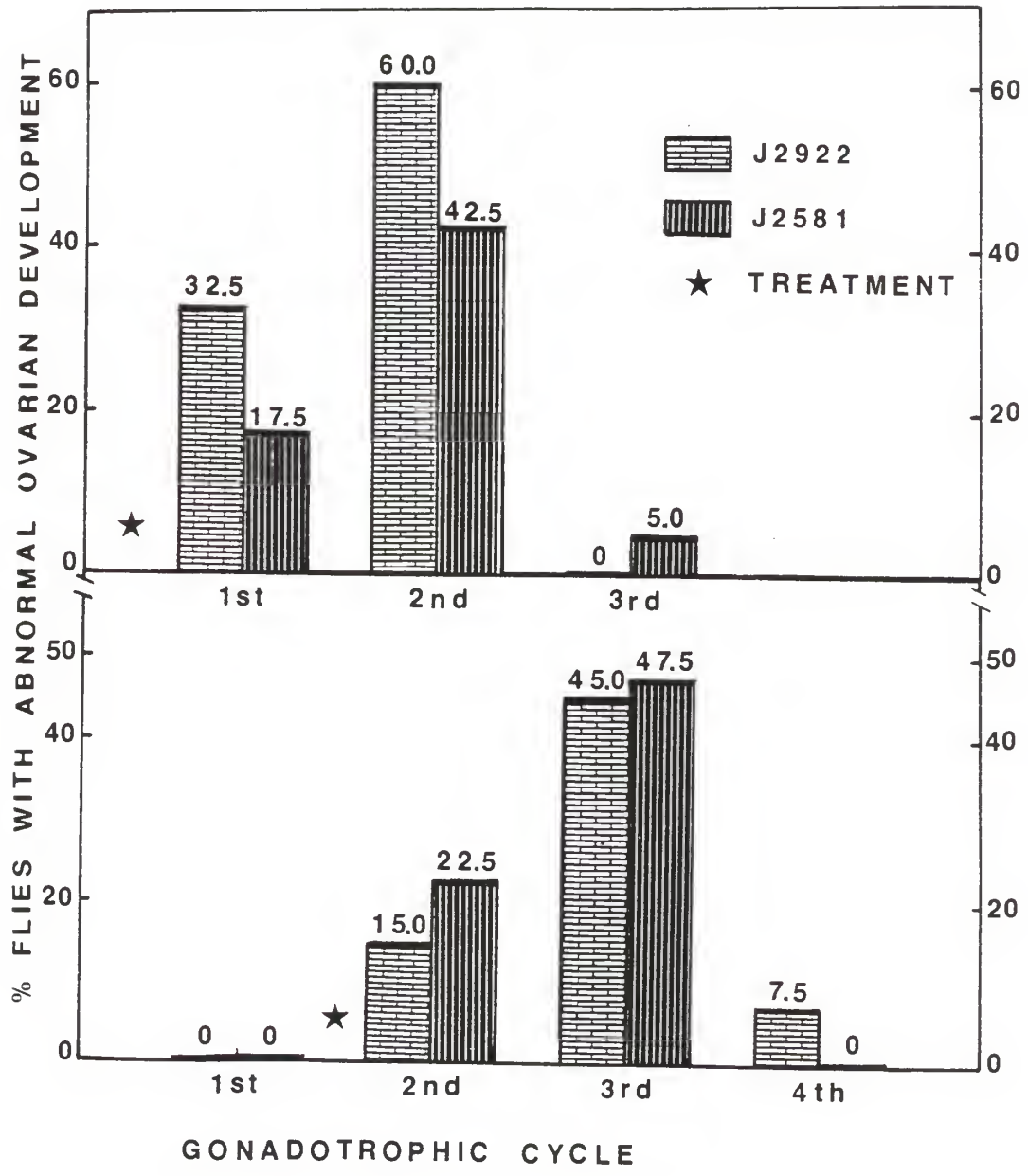
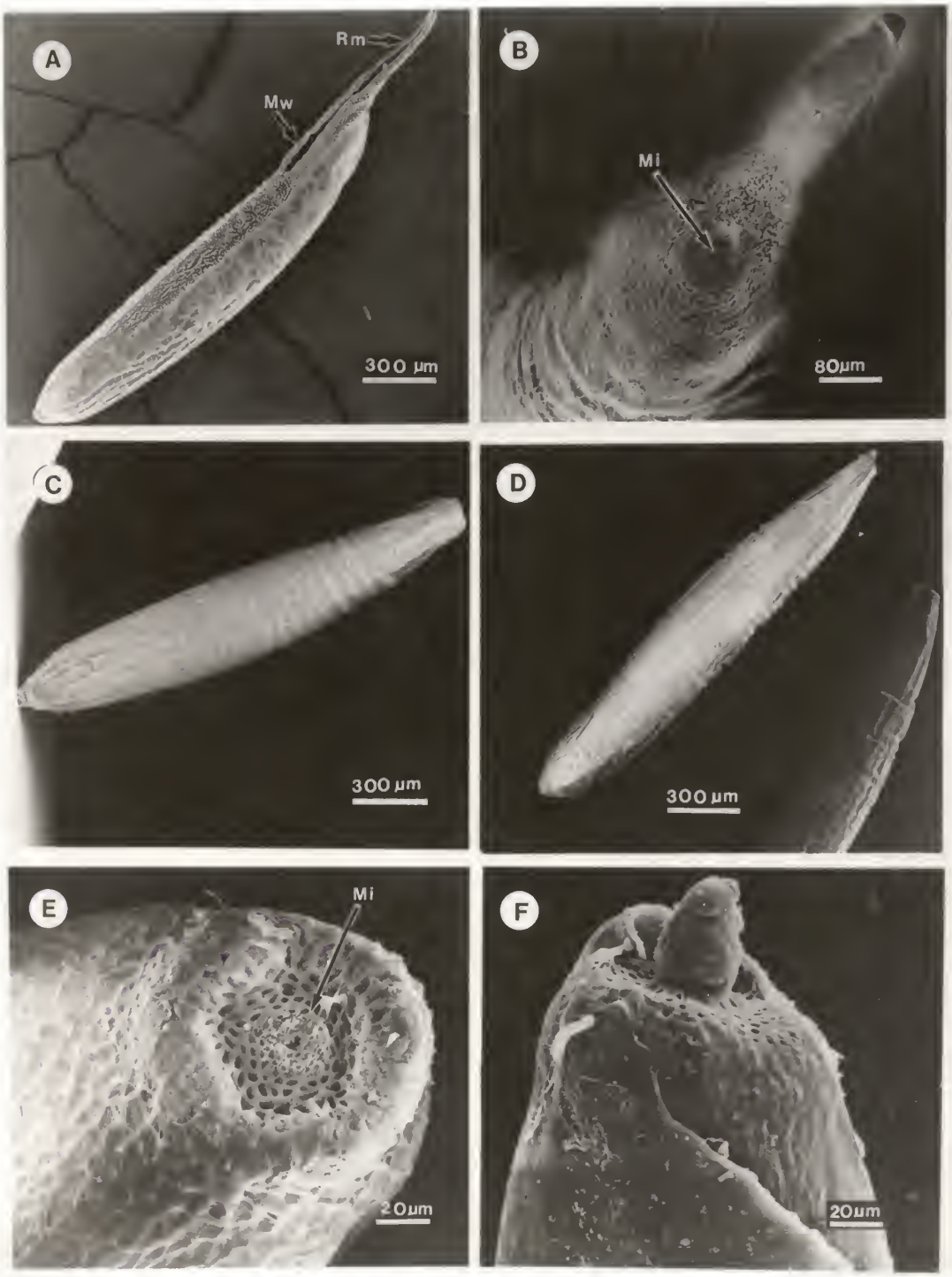

% FLIES WITH ABNORMAL OVARIAN DEVELOPMENT
60
40
20
0
32.5
17.5
60.0
42.5
0
5.0
J2922
J2581
TREATMENT
1st
2nd
3rd
50
40
20
0
0
0
15.0
22.5
45.0
47.5
7.5
0
1st
2nd
3rd
4th
GONADOTROPHIC CYCLE

Fig 2.-Eggs of face flies untreated and treated with J2922 and J2581 (0.1% AI). (A, B) normal eggs from untreated flies showing well developed respiratory mast (Rm), meshwork (Mw), and micropyle (Mi) located behind the respiratory mast. (C - F) eggs from treated females showing complete absence of respiratory mast and meshwork. Note micropyle on apical location (E) and micropyle degenerated (F).


A
Rm
Mw
300 µm
B
Mi
80µm
C
300 µm
D
300 µm
E
Mi
20µm
F
20µm

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Alberto B. Broce, for his invaluable guidance prior to and during the course of my study.

Much appreciation goes to the Fulbright Commission, Universidad Tecnica de Machala and the Latin American Scholarship Program of American Universities (LASPAU) for providing me the opportunity to continue my education in the United States.

Dr. R. G. Helgesen and the Department of Entomology were also instrumental in allowing me to further my studies.

Drs. Donald E. Mock and Gerald E. Wilde provided invaluable assistance by serving on my supervisory committee.

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EFFECTS OF SUBSTITUTED BENZYLPHENOLS AND SOME INSECT GROWTH REGULATORS ON THE REPRODUCTION OF FACE FLY, *Musca autumnalis* De Geer

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Entomology

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1984

ABSTRACT

New, non-mutagenic chemosterilants, a benzylphenol (J2644) and two benzyl-1,3-benzodioxoles (J2922 and J2581), and the insect growth regulators (IGR) BAY SIR 8514, CGA-72662 and Rol3-5223 were evaluated for their sterilizing effects on the face fly Musca autumnalis De Geer. J2644, J2922, J2581 and BAY SIR 8514 prevented egg hatching when flies were treated orally, topically or tarsally. J2644 and BAY SIR 8514 were not toxic at doses giving 100% inhibition of egg hatching and caused sterility even when flies were inseminated or near oviposition. Effects of J2922 and J2581 were similar in that their lowest doses giving maximum sterility were also highly toxic. Rol3-5223 was not effective at any of the tested concentrations (0.1 to 1.0% AI) administered orally or topically, nor was CGA-72662 applied orally.

J2644, J2922, J2581 and BAY SIR 8514 caused 100% sterility in females, whereas male fertility was only slightly reduced with J2644. The female sterility induced by these compounds was reversible. Flies treated with J2644, J2922 and J2581 restored their normal fertility by the 3rd gonadotrophic cycle, whereas flies treated with BAY SIR 8514 restored fertility by the 4th cycle. J2922 and J2581 affected egg hatching and ovarian development, observed as atrophied ovaries and ovaries containing eggs without respiratory mast, this effect was greater in the 2nd gonadotrophic cycle subsequent to the treatment. The effects on ovarian development observed with J2922 and J2581 may be useful in future physiological research and may help in understanding the mode of action of these compounds.